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THANGES IN THE HEMOLYMPH ACID-BASE STATE OF THE SHORE CRAB, CARCINUS MAENAS, EXPOSED TO SIMULATED TIDEPOOL CONDITIONS

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ABSTRACT

High-tide and low-tide conditions were established in an artificial pool containing algae by alternately circulating seawater and leaving it stagnant for successive 6-h periods. Diurnal cyclical changes of water temperature and partial pressures of oxygen and carbon dioxide were almost the same as in natural tidepools. Hemolymph acidbase status (pH, carbon dioxide partial pressure, and bicarbonate concentration) was determined during day and night low-tide periods in crabs (*Carcinus maenas*) acclimated to artificial pool conditions. Except at water P_{O_2} below 20 Torr where the animals breathed air and developed a partially compensated respiratory acidosis, acidbase disturbances induced by changes of respiratory gases were much less than those observed in single-factor laboratory experiments, mainly because of counteracting influences of oxygen and carbon dioxide. This resulted in a decrease of hemolymph pH at increasing water temperature of -0.016 pH unit $\cdot {}^{\circ}C^{-1}$, consistent with the imidazole alphastat hypothesis.

INTRODUCTION

Large and rapid fluctuations of many environmental factors are known to occur in small water bodies left on the shore by the receding tide (Newell, 1979; Truchot and Duhamel-Jouve, 1980; Morris and Taylor, 1983). In addition to variations of water temperature and salinity, the partial pressures and concentrations of respiratory gases, oxygen and carbon dioxide, may undergo conspicuous cyclic changes when animal and plant populations are present. During the day, photosynthesis usually surpasses respiratory activity, leading to an elevation of the water oxygen partial pressure (Pw_{O_2}) which may even be accentuated by a simultaneous increase of temperature. At the same time, carbon dioxide is depleted, water CO_2 partial pressure (Pw_{CO2}) decreases, and water pH (pHw) markedly increases. When tidepools are isolated from the open sea at low tide during the night, the opposite changes of Pw_{O_2} , Pw_{CO_2} , and pHw are observed in the absence of photosynthesis. Thus, at each tidal emersion cycle, these aquatic habitats become hypoxic-hypercapnic at night and hyperoxic-hypocapnic during the day. In addition, most probably because large changes of pH favor either precipitation or dissolution of calcium carbonate, the total titratable alkalinity tends to decrease during the day and to increase at night (Truchot and Duhamel-Jouve, 1980; Morris and Taylor, 1983).

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Abbreviations: α_{CO_2} : carbon dioxide solubility coefficient in blood; Cb_{CO2}: total carbon dioxide concentration in blood; Pb_{CO2}: carbon dioxide partial pressure in blood; pHb: blood pH; pHw: water pH; Pw_{O2}, Pw_{CO2}: oxygen and carbon dioxide partial pressures in water; TAw: water titration alkalinity.

All these factors acting separately are known to affect respiratory function and blood acid-base state of many aquatic animals (reviewed by Truchot, 1981a). For example, hypoxia induces an increase and hyperoxia a decrease of the ventilatory activity in fishes (Dejours, 1973), crustaceans (Jouve-Duhamel and Truchot, 1983), and annelids (Toulmond and Tchernigovtzeff, 1984). These ventilatory responses usually lead respectively to hypocapnic alkalosis and hypercapnic acidosis of the blood. Although ventilatory reactions to ambient CO₂ appear variable and their interpretation often controversial, numerous studies have unanimously demonstrated that an increased water P_{CO_2} induces a well-defined hypercapnic acidosis in aquatic animals. Acid-base responses to hypocapnic water, however, remain practically unknown, probably because of the difficulty of obtaining very low Pw_{CO_2} in laboratory conditions. Respiratory acidoses induced by either water hyperoxia or hypercapnia are usually compensated metabolically with an increase of the blood bicarbonate concentration, but these compensations develop slowly, taking many hours or days to be completed.

Obviously, the conditions to which intertidal animals may be exposed at low tide in rockpools are much more complicated than those examined up to now in laboratory experiments where the effects of each factor are studied separately. So many relevant factors vary simultaneously in natural tidepool conditions that the resulting changes of blood acid-base state are almost impossible to predict. The information available from laboratory studies is thus of limited significance in assessing physiological adaptation to this particular environment. In terms of survival value, the adaptational meaning of any physiological response to environmental changes can only be inferred from data obtained in ecologically realistic conditions.

According to the imidazole alphastat theory (Reeves, 1972, 1977), maintenance of an appropriate acid-base state in extra- and intracellular fluids is considered to be important in stabilizing macromolecular structure and function (Somero, 1981; White and Somero, 1982). This requires not only a constant pH at constant body temperature but also a temperature-induced change of pH of approximately -0.017 pH unit $\cdot {}^{\circ}C^{-1}$. Although many experimental data support this theory in various ectothermic animals, its general validity remains controversial. The required pH-temperature slope is not always found, particularly in aquatic animals (reviewed by Truchot, 1981a), and this failure often has been ascribed to a marked sensitivity of the acid-base status of waterbreathers to environmental conditions. Indeed, by separately manipulating various ambient factors in water at constant temperature, it is very easy to induce large and durable changes of blood pH without apparent harmful effects to the animals (see Dejours and Armand, 1982). Despite suggesting a wide tolerance of aquatic species to extracellular acid-base disturbances, such experiments tell us nothing about actual acid-base variations in animals subjected to simultaneous changes of temperature and many other factors in their natural environment.

The shore crab, *Carcinus maenas*, a mainly intertidal species on west European coasts, is often retained at low tide in residual water bodies on the shore. Much information has been previously acquired in the laboratory on the effects of various environmental factors on hemolymph acid-base state in this animal (Truchot, 1973a, b; 1975a, b; 1978; 1981b; 1984). In addition, changes of ventilatory activity have been directly recorded at low tide in natural rockpools (Jouve-Duhamel and Truchot, 1983). The main objective of this work was to determine how these various effects combine to set the crab's acid-base balance in natural conditions and particularly to assess the validity of the alphastat hypothesis in a continuously changing aquatic environment. Using an artificial system faithfully reproducing tidepools conditions, I describe the pattern of hemolymph acid-base changes induced by simultaneous variations of many environmental factors in this habitat.

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MATERIALS AND METHODS

Observations were performed during the summer (June and July 1985) when environmental fluctuations in tidepools are the greatest (Morris and Taylor, 1983). Male Gabs (*Carcinus maenas*), wet weight 30–50 g, collected near Arcachon, Southwest of France, were maintained at least one week before experiments in flowing seawater (temperature 19–21°C; salinity *ca.* 32‰) in the laboratory.

Artificial pool

Tidepool environmental conditions were established in an outdoor circular plastic tank of *ca.* 1.2 m diameter. Water volume was approximately 80 liter and the tilted bottom covered with a layer of gravel gave water depths between 3.5 and 9 cm, allowing the crabs to emerge spontaneously to breathe air, a behavior described by Taylor and Butler (1973). In the intertidal zone, natural rockpools are flooded at high tide and completely isolated from the open sea at low tide. Thus, to simulate high tide, water flowed through the tank at 1.1 liter $\cdot \min^{-1}$ during a 6-h period which was followed by a low tide 6-h period when the water circulation was stopped. Algae, mainly *Enteromorpha* spp., *Ulva* sp., and some *Fucus*, were introduced to obtain a moderately loose cover at the water surface. They grew rapidly, attaining a total wet mass of 1.8 kg at the end of the experiment. Flourishing copepod and amphipod (mainly *Hyale* spp.) populations probably introduced with the algae were also observed.

Ten to twelve crabs were acclimated for at least two high and low tide cycles in the tank before sampling, which was performed various times after the beginning of a low tide period, during the day or at night. During rainy periods, excessive water dilution was prevented by a transparent plastic cover which was not used on sunny days to avoid undue heating.

Water and blood sampling

Once a crab was located in the artificial pool, temperature was first recorded and a water sample was then drawn into a 20 ml syringe close to the inhalent openings at the base of the walking legs. The crab was then rapidly grasped before it could run away and a prebranchial hemolymph sample was obtained in a 1-ml syringe by puncturing the base of a walking leg. Water and hemolymph samples were used only when the animal had remained completely motionless before the catch. Only one hemolymph sample was obtained on each crab.

Measurements and calculations

After thermal equilibration of the syringe in a water bath, water pH (pHw) and P_{O_2} (Pw_{O2}) were measured with Radiometer electrodes at either 20, 22, or 25°C, according to the mean tank temperature expected for that particular day. The pHw values were corrected to the *in situ* temperature using temperature coefficients $\Delta pH/\Delta t$ determined in preliminary experiments on seawater samples kept anaerobically in closed syringes ($\Delta pHw/\Delta t = -0.013$ and -0.010 pH unit $\circ C^{-1}$ at pH values above and below 8.0, respectively). Pw_{O2} values were similarly corrected using oxygen solubility coefficients at various temperatures and salinities (Riley and Skirrow, 1975). Water salinity was deduced from chloride concentration measured with a Radiometer CMT 10 Chloride Titrator. Total titratable alkalinity, TAw, was determined by Gran titration following a procedure outlined by Culberson *et al.* (1970). From pHw and TAw measurements, water P_{CO2} (Pw_{CO2}) was calculated as described by Truchot and Duhamel-Jouve (1980).

On each hemolymph sample, pH (pHb) was immediately measured at either 20, 22, or 25°C with a Radiometer G297/G2 pH microelectrode and was corrected to the *in situ* temperature using a $\Delta pHb/\Delta t$ coefficient of -0.0195 pH unit °C⁻¹, valid for *Carcinus* blood kept anaerobically in a closed syringe (Truchot, 1973a). Total hemolymph carbon dioxide concentration (Cb_{CO2}) was measured by the method of Cameron (1971). From measured pHb and Cb_{CO2}, and from the solubility coefficient α_{CO2} and carbonic acid dissociation constants K'₁ and K'₂ at the prevailing temperature and salinity (Truchot, 1976a), the hemolymph P_{CO2} (Pb_{CO2}) and (bicarbonate + carbonate) concentration were calculated for each sample using the formulas:

$$Pb_{CO_2} = Cb_{CO_2} / \alpha_{CO_2} \left(1 + \frac{K_1'}{10^{-pH}} + \frac{K_1'K_2'}{(10^{-pH})^2} \right)$$
$$[HCO_3^- + CO_3^{2-}](meq \cdot L^{-1}) = \alpha_{CO_2} \cdot Pb_{CO_2} \left(\frac{K_1'}{10^{-pH}} + \frac{2K_1'K_2'}{(10^{-pH})^2} \right)$$

Unless otherwise stated, results are presented as mean values \pm S.E.M. Statistically significant differences between mean values are reported at the *P* < 0.05 level.

RESULTS

General observations

Measurements were performed on 72 crabs during 6 daylight and 2 night experimental sessions. After switching to low tide conditions, the water usually became hyperoxic-hypocapnic in the daytime and hypoxic-hypercapnic at night. On one day session, however, the weather was very cloudy and both hypoxic and hyperoxic conditions were observed, depending on the time of the day.

The time-courses of the changes of the environmental factors were very similar to those recorded in natural rockpools in Roscoff, Brittany, France, during a previous study (Truchot and Duhamel-Jouve, 1980). Since water samples were taken only in the near vicinity of the crab to be subjected to hemolymph sampling, possible inhomogeneities in the water mass could not be documented. However, these inhomogeneities were most likely weak, since the various parameters measured on samples from different locations in the tank usually changed evenly with time. An example is shown in Figure 1 for a daylight and for a night low-tide period. During the day, Pw_{02} and pHw increased, and Pw_{CO2} and TAw decreased; converse changes were observed at night. The only differences from previous observations concern the higher temperature range and the lower salinities, mainly because of the more southern location. On sunny days, temperature increased strongly up to 30°C, but decreased almost imperceptibly at night because air temperature was relatively high. Salinity changes were moderate, no more than 1‰ during an experimental session. A comparison of average extreme values recorded in the artificial pool after 4–6 h low tide with those observed in natural rockpools in Roscoff at the same season is shown in Table I. The striking similarity of the ranges indicates that tidepool conditions were successfully established in the artificial enclosure.

Crabs put in the tank exhibited first an exploratory behavior, walking around for some time and then remaining quietly hidden within the algal mat. They were agitated only at night when the water became hypoxic. At a Pw_{O_2} around 20 to 30 Torr, they typically exhibited an emersion behavior, raising the anterior border of the cephalothorax above the water level and taking air through the normally exhalent apertures,



FIGURE 1. One example of the changes of temperature, salinity (S‰), pH, titration alkalinity (TAw), and partial pressures of oxygen (Pw_{O_2}) and carbon dioxide (Pw_{CO_2}) recorded in the artificial tidepool as a function of time elapsed after stopping water circulation during daytime or at night. Each data point at a given time corresponds to a water sample taken just before removing a crab from the tank for hemolymph sampling.

TABLE I

	Artificial pool (Arcachon)		Natural rockpools (Roscoff)	
	Night $(n = 2)$	$\begin{array}{l} \text{Day} \\ (n = 6) \end{array}$	Night $(n = 4)$	Day (n = 4)
t (°C)	20.4	25.4	12.4	23.2
	()	(2.2)	(0.2)	(0.6)
S (%O)	30.3	31.3	34.5	36.2
	(—)	(0.3)	(0.1)	(0.2)
P _{o2} (Torr)	6.3	447	21.4	495
	(—)	(48)	(12.0)	(28)
P _{co2} (Torr)	2.56 (—)	$2.17 \cdot 10^{-2} \\ (2.05 \cdot 10^{-2})$	1.77 (0.23)	$8.0 \cdot 10^{-4}$ (2.8 \cdot 10^{-4})
рН —	7.33	9.33	7.49	9.82
	(—)	(0.20)	(0.05)	(0.09)
TA (meq $\cdot L^{-1}$)	2.25	1.92 (0.09)	2.46 (0.01)	2.05 (0.08)

Average extreme values recorded after 4–6 h emersion in the artificial pool, compared to similar data at the same season (June–July) in natural rockpools on a more northern shore at Roscoff (from Truchot and Duhamel-Jouve, 1980)¹

¹ Mean values (S.E.M. in parentheses) from four different pools in Roscoff, and from two night and six daytime experimental sessions in the artificial pool.

as described by Taylor and Butler (1973). They generally reimmersed quickly when disturbed by an observer. Samples could be taken from these animals only when they were under water but frequent appearance of air bubbles indicated that most probably all immersed crabs had air in their gill chambers in these conditions.

Hemolymph acid-base values

Since the time-courses of the changes of the various environmental factors were different from day to day because of variable climatic conditions, presentation of the data as a function of the time elapsed from the beginning of a low-tide period would bear little meaning. Similarly, temperature changes were not reproducible from a given session to another. The conditions in this very variable environment are best described by the changes of respiratory gases and, for this reason, the acid-base values are presented according to the Pw_{O_2} range prevailing at the time of measurement. In Table II, seven Pw_{O_2} intervals, three in the hypoxic region, one normoxic, between 100 and 200 Torr, and three in the hyperoxic region are given. Except for the one below 20 Torr, corresponding to crabs predominantly breathing air, these classes are arbitrarily defined, but other limits do not modify the general pattern observed.

In moderately hyperoxic conditions, mean blood pH tended to decrease, albeit weakly, and remained steady or even increased when the water became strongly hyperoxic. Despite very low water P_{CO_2} , this tendency corresponded to Pb_{CO_2} values significantly elevated compared to those found in the normoxic range. Conversely, blood pH showed a trend to increase in the hypoxic range, at least down to 20 Torr

				Pw ₀₂ range (Torr)			
	<20	20-50	50-100	100-200	200–350	350-500	>500
u	11	9	10	7	10	24	4
Mean Рw _{o2} (Тогг)	11.1 (1.2)	37.1 (3.7)	77.4 (5.7)	135 (11)	286 (20)	448 (7)	533 (5)
Mean Pw _{co2} (Torr)	1.96 (0.20)	0.99 (0.08)	0.60 (0.04)	0.38 (0.05)	0.11 (0.02)	0.01 (0.002)	0.002 (0.001)
Mean pHw	7.46 (0.05)	7.72 (0.03)	7.92 (0.02)	8.11 (0.05)	8.55 (0.05)	9.29 (0.06)	9.57 (0.07)
Mean temp. (°C)	20.7 (0.4)	20.9 (0.5)	21.4 (0.3)	21.4 (0.2)	23.7 (0.7)	26.7 (0.5)	28.8 (0.3)
dHb	+7.711 (0.026)	7.843 (0.032)	7.778 (0.033)	7.776 (0.046)	7.746 (0.032)	*7.703 (0.015)	7.736 (0.027)
Pb ₆₀₂ (Torr)	**4.82 (0.48)	2.00 (0.32)	1.84 (0.16)	1.71 (0.17)	**2.49 (0.20)	**2.42 (0.09)	*2.27 (0.10)
$[HCO_{3}^{-} + CO_{3}^{2-}]$ $(meq \cdot L^{-1})$	**11.20 (1.25)	6.74 (1.59)	5.35 (0.86)	4.87 (0.90)	5.97 (0.38)	5.11 (0.22)	5.06 (0.13)

TABLE II

(*) Data classified by range of water oxygen tension recorded. S.E.M. in parentheses.

* Significantly different at the P < 0.05 (*) or P < 0.01 (**) levels from the values recorded in the normoxic range (Pw₂₀ = 100-200 Torr). \ddagger Significantly different (P < 0.01) from the pHb value recorded in the 20–50 Pw₆₂ range.

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 Pw_{O_2} , but blood P_{CO_2} did not significantly change. (Bicarbonate + carbonate) concentration did not change significantly from 20–50 Torr to >500 Torr Pw_{O_2} , remaining around 5–6 meq $\cdot L^{-1}$.

At Pw_{O_2} values below 20 Torr, when the crabs were likely breathing air during the night low-tide period, there was a steep significant decrease of blood pH and a strong increase of Pb_{CO_2} up to 4.8 Torr. This typical respiratory acidosis was acompanied by a marked increase of the blood (bicarbonate + carbonate) concentration.

DISCUSSION

Even though an artificial enclosure was used as a simulated tidepool, present data can be considered as representative of responses occurring in natural pool conditions at low tide on the shore. The reconstituted habitat was maintained with growing seaweed and flourishing animal life during more than one month. Furthermore, time-courses and extreme values of the relevant environmental variables, mainly temper-ature, Pw_{O_2} , and Pw_{CO_2} , revealed important fluctuations which were almost the same as those recorded in natural rockpools at the same season on west European coasts (Truchot and Duhamel-Jouve, 1980; Morris and Taylor, 1983).

According to previously published laboratory data (Truchot, 1973a; 1975a, b), if such large changes of physicochemical factors had been considered separately, there would have been important acid-base disturbances in *Carcinus maenas*. The most striking finding in this study is the modest variation of pHb and Pb_{CO_2} recorded in near natural conditions. Except in very hypoxic water at night, mean blood pH and P_{CO_2} ranged respectively from 7.70 to 7.85 and from 1.7 to 2.4 Torr.

The most important factors to be considered in interpreting these acid-base changes are the variations of the temperature and of the partial pressures of oxygen and carbon dioxide in the water. Salinity, which affects acid-base balance in *Carcinus maenas* (Truchot, 1973b; 1981b), changed too little to matter. The day-time decrease of seawater titratable alkalinity and its increase at night were too small to be implicated (Truchot, 1984).

Although acid-base disturbances were considerably damped in this study compared to previous laboratory findings, some well-defined trends can be discerned. In field conditions, *Carcinus maenas* is known to hypoventilate its gills during the day in hyperoxic-hypocaphic water (Jouve-Duhamel and Truchot, 1983). Accordingly, a slight hypercapnic acidosis did occur here during low tide in the daytime. However this disturbance was very limited, probably because of the simultaneous progressive lowering of Pw_{CO2} . One initial purpose of this work was the study of the acid-base state of crabs in extreme hypocapnic conditions, which could not be reproduced in the laboratory. That the animals could maintain near-normal blood pH and P_{CO_2} values in this situation was unexpected, but resulted simply from the concomitant hypoventilation in hyperoxia. In the hypoxic range, there was a trend to a blood alkalosis, reminiscent of the internal hypocapnia usually associated with the hyperventilatory reaction induced by ambient hypoxia. However, the present data show no sign of decreased blood P_{CO_2} , probably because the water P_{CO_2} concomitantly increased in natural conditions. On the whole, at least for crabs relying only on water breathing at Pw_{0} , values above 20 Torr, the pattern observed may be mainly explained by the counteracting effects of ambient Pw_{O_2} and Pw_{CO_2} changes, resulting in relatively limited acid-base disturbances. This is illustrated in Figure 2, which compares blood pH and P_{CO_2} values of this study with those from previous laboratory experiments at variable



FIGURE 2. Mean hemolymph pH and P_{CO_2} values recorded in this study and in previous laboratory experiments on the crab *Carcinus maenas*. Data are plotted as a function of water P_{O_2} together with corresponding mean Pw_{CO_2} and temperature values recorded in the artificial pool. \bullet = data from this study at variable Pw_{CO_2} and temperature; O = data at variable Pw_{O_2} and constant temperature (17°C) and Pw_{CO_2} (about 0.3 Torr) (from Truchot, 1975a).

 Pw_{O_2} but constant Pw_{CO_2} and temperature. It is apparent that the respiratory alkalosis usually recorded in normocapnic-hypoxic water is limited by the concomitant external hypercapnia observed in tidepool conditions. Similarly, in the hyperoxic range, the

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hypercapnic acidosis linked to the depressed ventilation remains moderate because the water becomes hypocapnic in the natural setting.

What happens in severe hypoxia at $Pw_{CO_2} < 20$ Torr requires special comment. The findings confirm previous observations in showing that partially emerged crabs taking air into their gill chamber rapidly developed a hypercapnic acidosis (Wheatly and Taylor, 1979). This particular respiratory mode is advantageous in terms of oxygen acquisition (Taylor and Butler, 1973; Taylor *et al.*, 1977) and present data indicate that the associated acid-base disturbance remains moderate. In near natural conditions, we observed the emersion response at relatively low ambient Pw_{O_2} ($\simeq 20$ Torr at 20– 21°C), much lower than in the laboratory observations of Taylor *et al.* (1977) (42 Torr at 17–18°C) and Wheatly and Taylor (1979) (51 Torr at 20°C). Whether this disagreement results from other factors, such as the Pw_{CO_2} level, remains to be studied. A related point is that crabs never did emigrate out of the water at high temperature, as observed by Taylor and Wheatly (1979). Since high water temperature was always associated with hyperoxia in the artificial pool, it may be that the emigration behavior is concerned more with oxygen acquisition than with heat avoidance.

Laboratory observations have shown that hypercaphic acidosis induced either by ambient hyperoxia, hypercapnia, or air breathing is partially compensated metabolically only after at least 10 hours in Carcinus maenas (Truchot, 1975a, b, c). Thus an important question is whether metabolic compensations occur in natural conditions. limiting acid-base disturbances during rapid environmental changes. The present data do not show any significant differences in the hemolymph bicarbonate concentrations for any Pw₀₂ value above 20 Torr, suggesting that the relevant environmental factors change too rapidly to allow completion of appropriate compensations. However, the increase of bicarbonate concentration below $Pw_{O_2} = 20$ Torr appears too large to be accounted for by passive physico-chemical buffering in the hemolymph. In fact, the apparent buffer slope, $-\Delta[HCO_3^- + CO_3^2]/\Delta pH$, calculated between mean observed values in the 20–50 and <20 Torr Pw₀, ranges was 33.8 meq \cdot L⁻¹ \cdot (pH unit)⁻¹, considerably higher than the usual *in vitro* buffering capacity of *Carcinus* hemolymph (Truchot, 1976b). This indicates that the hypercapnic acidosis is at least partially compensated metabolically, a process which may be more effective in *in situ* than in laboratory conditions, as is also suggested by observations on another crab, *Cancer* productus (De Fur et al., 1983).

Present data must be now discussed within the framework of the imidazole alphastat theory of acid-base regulation in animals (Reeves, 1972; 1977). This theory contends that to stabilize protein function, intracellular pH should change with body temperature at a rate of -0.017 pH unit $\cdot \circ C^{-1}$. It would obviously be advantageous for this intracellular homeostasis that extracellular pH changes with the same slope. That this is not always found in laboratory investigations on aquatic animals has been taken as evidence against the theory (for example Heisler, 1984). However, as stressed by White and Somero (1982), the thermal regimens customarily established in laboratory acclimation experiments can be very different from those encountered by animals in their natural environments. By design, laboratory experiments usually consider the effects of temperature changes with other factors held constant, particularly oxygen and carbon dioxide partial pressures. In the natural aquatic environment, this design mainly simulates seasonal temperature variations in large water masses, oceans, or lakes. Such changes are very much slower than those ordinarily imposed in laboratory experiments and they may allow for the development of many additional adaptive processes (White and Somero, 1982). By contrast, rapid temperature variations usually occur on an hourly or diurnal basis in smaller water bodies where they are most



FIGURE 3. Individual pH values measured on 61 crabs *Carcinus maenas* breathing water in the artificial pool in low-tide conditions, plotted as a function of water temperature. Open symbol with error bars represents the mean pH value for 11 crabs breathing air at $Pw_{O_2} < 20$ Torr. Regression line calculated for values of crabs breathing water only. Correlation coefficient r = -0.54; P < 0.001.

frequently accompanied by large changes of oxygen and carbon dioxide partial pressures. This is the case in tidepools and the present data thus offer the opportunity to test the imidazole alphastat theory in ecologically realistic conditions.

Figure 3 shows a plot of individual pH data collected in this study as a function of temperature for crabs breathing water at $Pw_{O_2} > 20$ Torr. The scatter of these data is much larger than usually found in laboratory experiments. This is not surprising because environmental changes were not exactly the same from one low tide session to another and because different Pw_{O_2} and Pw_{CO_2} levels thus prevailed at a given temperature in the artificial pool. However, the pH-temperature slope calculated by linear regression amounts to -0.0157 pH unit $\cdot ^{\circ}C^{-1}$ in the range 20 to 30 °C with a highly significant correlation coefficient (r = -0.54; P < 0.001). The slope value fits the imidazole alphastat theory satisfactorily and approaches that required for a constant relative alkalinity (-0.0167 in the same temperature range). Practically the same slope was observed previously in laboratory experiments at constant water P_{O_2} and P_{CO_2} in crabs *Carcinus* acclimated several days at various temperatures, (Truchot, 1973a; Dejours *et al.*, 1985).

Customarily it is thought that pH-temperature adjustments in aquatic animals rely on changes of the blood bicarbonate concentration and thus require a relatively long acclimation period. The present data suggest that the same result can be attained rapidly in natural conditions when water P_{O_2} and P_{CO_2} simultaneously change with temperature. Whether this represents a particular adaptive pattern in intertidal rockpool animals awaits further studies. But, since no changes of hemolymph bicarbonate concentration are apparent in tidepool conditions, it may well be that the observed pattern results not from specific compensatory processes but simply from usual ventilatory responses to the concerted variations of P_{O_2} and P_{CO_2} occurring in this rapidly changing aquatic environment.

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